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Profile and prognostic value of tumour-infiltrating lymphocytes in triple-negative breast cancer in women in Kinshasa at CUK, Democratic Republic of the Congo

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ABSTRACT

Introduction

Triple-negative breast cancer (TNBC), known for its particularly aggressive nature, is associated with a poor prognosis. Tumour-infiltrating lymphocytes (TILs) are emerging as a key prognostic biomarker in this subtype.

Purpose

To quantitatively analyse TILs and explore their association with clinical and pathological characteristics among Congolese patients.

Methods

A cross-sectional, descriptive study was conducted between January 2022 and December 2024 in two reference cytopathology laboratories in Kinshasa (University Clinics and LEBOMA Pathology Cabinet). A convenience sample of 147 breast cancer patients was included, of whom 28 had triple-negative breast cancer confirmed by immunohistochemistry. Data were extracted using Microsoft Excel 2016 and analysed with SPSS version 27. Proportion comparisons were performed using the chi-square test, with statistical significance set at $p < 0.05$.

Results

Among the 147 breast cancer patients included, 28 (19.05%) had TNBC. The intermediate TIL group was the most represented (39.3%), predominantly associated with grade 3 tumours, followed by the low TIL group (35%), where grade 2 tumours predominated (60%). The age group ≤ 50 years accounted for 19 patients (67.9%), among whom 9 (47.3%; 95% CI [27.3–68.3]) had intermediate TILs. A low degree of TILs was observed in 6 patients (66.7%) aged > 50 years. A Ki-67 proliferative index > 30% was noted in 21 patients (75%), of whom 10 (47.6%; 95% CI [28.3–67.6]) had intermediate TILs.

Conclusion

The majority of TNBC patients had TIL levels ≥10. High TIL density was associated with younger age and a lower Ki-67 proliferation index. TIL assessment therefore emerges as a relevant prognostic biomarker that could guide patient follow-up and therapeutic decision-making, particularly regarding immunotherapy.

INTRODUCTION

Breast cancer is a major public health concern, with approximately 2.3 million new cases annually, representing 11.6% of all cancers and making it the leading cause of cancer-related death among women (Bray et al., 2024; World Health Organization [WHO], n.d.). Although the incidence is higher in developed countries, mortality is paradoxically greater in sub-Saharan Africa due to late diagnosis and limited access to care (DeSantis et al., 2019; Gnangnon, 2024).

In the Democratic Republic of the Congo (DRC), the absence of a national cancer registry hinders reliable data collection. Nevertheless, available studies indicate that breast cancer is now the most frequent malignancy among women (Nguala et al., 2021; Katumbayi et al., 2022).

Breast cancer exhibits marked morphological and molecular heterogeneity. Among Congolese women, the luminal A subtype is the most common, followed by luminal B, while triple-negative breast cancer (TNBC) accounts for approximately 10% of cases (Sulu et al., 2022). TNBC is particularly aggressive and associated with poor prognosis, underscoring the need for accessible biomarkers to improve patient stratification and guide therapeutic decisions.

Tumour-infiltrating lymphocytes (TILs) are recognised as prognostic and predictive biomarkers in TNBC, with their density associated with immune response, age, Ki-67 proliferation index, and survival outcomes (Denkert et al., 2018; de Jong et al., 2022; Loi et al., 2014; Loibl et al., 2022). In resource-limited settings such as the DRC, quantitative assessment of TILs may offer a simple and reliable approach to refine prognosis and inform treatment strategies, including potential immunotherapy.

The objective of this study was to determine the quantitative profile of TILs and to examine their association with clinicopathological characteristics in a cohort of Congolese patients with TNBC, with the aim of enhancing diagnostic strategies and assessing, through this biomarker, the prognosis of this poorly studied cancer subtype in our local setting.

METHODS

Type of Study, Period, Setting, and Study Sample

This cross-sectional, descriptive study was conducted from January 2022 to December 2024 in two reference cytopathology laboratories in Kinshasa, DRC: the Department of Pathological Anatomy at the University Clinics of Kinshasa and the LEBOMA Laboratory. The study population included patients diagnosed with breast cancer who had undergone immunohistochemical analysis. A non-probabilistic convenience sample of 147 patients was selected, 28 of whom had TNBC.

Inclusion Criteria

All cases of TNBC for which haematoxylin–eosin-stained blocks and slides were available in the archive, allowing review and confirmation of diagnosis by two pathologists, were included.

Exclusion Criteria

Cases of TNBC for which blocks and slides were missing or could not be retrieved were excluded.

Variables of Interest

Data were recorded in an electronic form (KoboCollect) that included age and clinicopathological characteristics (Ki-67 proliferation index, SBR grade, and degree of TILs).

Data Collection

Registers and immunohistochemical protocols from the above-mentioned departments were reviewed to identify patients with TNBC. The blocks and haematoxylin–eosin-stained slides were then retrieved from the archives for review and diagnostic confirmation by two pathologists. Re-cuts were performed for cases with poorly legible slides.

Histopathological Laboratory Equipment and Techniques

- Tissue sections were stained with haematoxylin–eosin for histopathological evaluation.
- For each TNBC case, diagnosis was confirmed by re-examining archived slides and blocks.
- Hormone receptor negativity and Ki-67 proliferation index were assessed using the Benchmark GX IHC/ISH automated system (Roche) with the following antibodies:

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- Anti-oestrogen receptor (ER) (SP1) rabbit monoclonal primary antibody.
- Anti-progesterone receptor (PR) (1E2) rabbit monoclonal primary antibody.
- Anti-HER2/neu (4B5) for HER2 receptor.
- Anti-Ki-67 (30-9) rabbit monoclonal primary antibody for proliferation index.

- Quantitative assessment of TILs was performed on haematoxylin–eosin-stained slides by determining the percentage of mononuclear inflammatory cells within the total tumour stromal area, following the guidelines of Salgado et al. (2015).

Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 27 after extraction into Excel 2016. Qualitative variables were summarised as frequencies and percentages, and quantitative variables as means and standard deviations. Results were presented in figures and tables. Proportion comparisons were performed using the chi-square test, with significance set at $p < 0.05$.

Ethical Principles

Since this study was based exclusively on archived data without direct interaction with patients, it was not necessary to obtain ethical approval. However, the principles of anonymity and confidentiality were strictly observed.

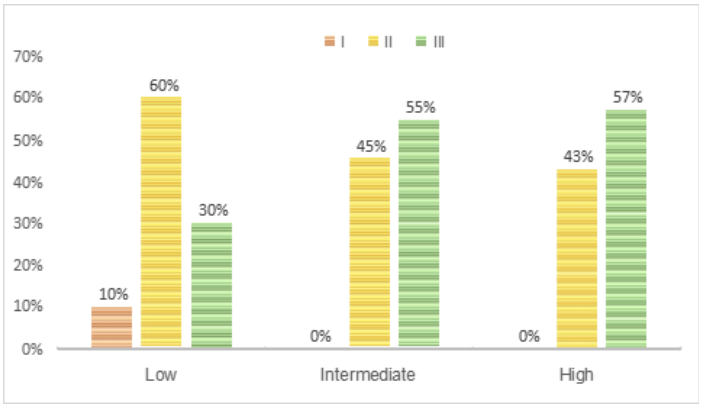
RESULTS

Of the 147 breast cancer patients analysed, 28 (19.05%) had TNBC.

Degree of TILs

The intermediate TIL group was the most represented, observed in 11 patients (39.3%), with most classified as SBR grade 3 (6 cases, 54.5%). The low TIL group included 10 patients (35.7%), of whom 6 were SBR grade 2 (60%). The high TIL group was identified in 7 patients (25%), with 4 (57.1%) being SBR grade 3.

Figure 1:
Distribution of patients by degree of TILs and SBR grade



TILs, Age, and Ki-67 Proliferative Index

The age group ≤ 50 years included 19 patients (67.9%), of whom 9 (47.3%; 95% CI [27.3–68.3]) presented intermediate TILs. However, this difference did not reach statistical significance ($p = 0.062$). A low degree of TILs was observed in 6 patients (66.7%) older than 50 years. A Ki-67 index > 30% was found in 21 patients (75%), of whom 10 (47.6%; 95% CI [28.3–67.6]) had intermediate TILs ($p = 0.065$).

Table 1:
Distribution of Age and Ki67 by Category (N = 28)

Variable	Category	Low *n (%)*	Intermediate *n (%)*	High *n (%)*	Total
Age	≤ 50	4 (21.1%)	9 (47.4%)	6 (31.6%)	19
	> 50	6 (66.7%)	2 (22.2%)	1 (11.1%)	9
Ki67	< 14	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
	14–30	2 (28.6%)	1 (14.3%)	4 (57.1%)	7
	> 30	8 (38.1%)	10 (47.6%)	3 (14.3%)	21

DISCUSSION

The prevalence of TNBC in this cohort (19.05%) aligns with data reported among Black women. The American Cancer Society notes that TNBC accounts for about 19% of breast cancer diagnoses among Black women in the United States, compared with 9–11% among other ethnic groups (Giaquinto et al., 2024). However, our findings are lower than those of some African studies, including in Benin, where higher rates have been reported (Gnangnon, 2024). These discrepancies may be due to methodological differences such as variability in hormone receptor assessment, sample size, and genetic diversity across populations.

The predominance of intermediate and low TILs in our study supports the findings of Feng et al. (2021) and de Jong et al. (2022), who demonstrated that high TIL density is mainly associated with high-grade tumours. This suggests that a more pronounced immune response is often mounted against biologically aggressive tumours, although this response does not necessarily translate into improved prognosis.

Age also appears to influence TIL density. Our results are consistent with those of Takada et al. (2022), who reported reduced TIL infiltration in older patients. This reduction can be attributed to immunosenescence, characterised by decreased activity of T lymphocytes—particularly cytotoxic $CD8^+$ T cells—which limits tumour cell recognition and elimination (Goronzy et al., 2009). Additionally, ageing alters the tumour microenvironment by disrupting cytokine and chemokine production, which are essential for lymphocyte recruitment (Santhanam et al., 2018).

The observed association between high Ki-67 proliferation (>30%) and low TILs aligns with conclusions from the French National Cancer Institute (Thesaurus, 2024), identifying this combination as an indicator of high tumour aggressiveness and poor prognosis in TNBC.

The link between TILs and Ki-67 reflects a biologically plausible interaction: rapidly proliferating tumours tend to be more immunogenic due to increased mutational load and neoantigen presentation, promoting immune recognition (Denkert et al., 2018). Moreover, inflammation driven by tumour proliferation can enhance immune cell recruitment (Grivennikov et al., 2010). Wang et al. (2021) similarly found that high post-chemotherapy TIL density was associated with lower Ki-67 expression and better prognosis.

From a clinical perspective, TIL density may represent a valuable prognostic and predictive biomarker in low-resource settings such as the DRC. Unlike molecular profiling—which is costly and often unavailable—TIL assessment can be performed on routine histological slides at minimal cost. Incorporating TIL evaluation into routine pathology could therefore enhance prognostic stratification and inform treatment decisions in TNBC.

CONCLUSION

This study demonstrates that a TIL density ≥10% is common in TNBC in Kinshasa, particularly among younger patients and those with higher Ki-67 indices, though not significantly correlated with tumour grade. These findings reinforce the role of TILs as prognostic and predictive biomarkers. Their systematic inclusion in pathology reports could improve prognostic accuracy and inform immunotherapy decisions. Nevertheless, the retrospective design and lack of qualitative TIL analysis limit generalisability, warranting prospective, multicentre studies incorporating immune phenotyping.

In the DRC, developing national guidelines for TIL quantification, establishing a TNBC registry, and strengthening pathologist training are crucial steps toward improving breast cancer management in resource-limited settings.

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Conflicts of Interest: None declared.

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